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High Frequency Expressions of CD44 Standard and Variant Forms in Non-Small Cell Lung Cancers, But Not in Small Cell Lung Cancers

KAZUYA KONDO, MD, PhD,[1]* TAKANORI MIYOSHI, MD, PhD,[1] NAOKI HINO, MD, PhD,[1] EIJI SHIMIZU, MD, PhD,[2] NORIYUKI MASUDA, MD,[3] MINORU TAKADA, MD,[3] TADASHI UYAMA, MD, PhD,[1] AND YASUMASA MONDEN, MD, PhD[1]

[1]Second Department of Surgery, School of Medicine, University of Tokushima, Tokushima City, Tokushima, Japan
[2]Third Department of Internal Medicine, School of Medicine, University of Tokushima, Tokushima City, Tokushima, Japan
[3]Department of Internal Medicine, Osaka Prefectural Habikino Hospital, Habikino, Osaka, Japan.

Background and Objectives: Organ specificity has been demonstrated in the mode of CD44 expression among several cancers.
Methods: We examined the expressions of CD44 standard (CD44s) and CD44 variants (CD44v) in 14 cell lines (small cell lung cancer (SCLC): 5, non-small cell lung cancer (NSCLC): 9 and 20 surgically resected samples (SCLC: 7, NSCLC: 13) of lung cancer using reverse transcription-polymerase chain reaction and immunohistochemistry.
Results: Although both NSCLC and SCLC expressed CD44s, the frequency and intensity of CD44s expression in NSCLC were different from those in SCLC: cell lines, 89% vs. 40%; tumor samples, 100% (diffusely stained) vs. 57% (focally stained). CD44s expression was partially or completely repressed in SCLC. However, NSCLC frequently expressed CD44v, but SCLC expressed infrequently: cell lines, 67% vs. 20%; tumor samples, 69% vs. 0%. The N-417 line, which only expressed some CD44v in SCLC, falls SCLC and NSCLC both in biomarkers and in growth patterns.
Conclusions: CD44 expression was repressed in SCLC but was enhanced in NSCLC. *J. Surg. Oncol. 1998;69:128–136.*

KEY WORDS: RT-PCR; immunohistochemistry; CD44 repression; growth pattern

INTRODUCTION

CD44 is an integral membrane glycoprotein widely distributed on various types of cells; it functions as a lymphocyte homing receptor for circulating lymphocytes and as an adhesion molecule for epithelial cells [1–4]. It appears in various isoforms that consist of CD44 standard (CD44s) or CD44 variants (CD44v) generated by the alternative splicing of up to 10 exons [5].

Recent studies have reported that the CD44v isoforms are sufficient to confer metastatic potential to low or nonmetastatic rodent cancer cells [6] and that they are related to tumor progression and aggressiveness in a variety of human cancers [6–11]. There is currently a great deal of interest in the roles of a certain form of CD44v in tumor progression in several cancers.

Previous studies have shown that CD44s and CD44v were frequently expressed both in cell lines and in human

*Correspondence to: Kazuya Kondo, MD, Second Department of Surgery, School of Medicine, University of Tokushima, Kuramoto-cho, 2 chome, Tokushima City, Tokushima, 770-8503, Japan. Fax No.: (81)886-33-7144. E-mail: kondo@clin.med.tokushima-u.ac.jp
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TABLE I. Characteristics of Lung Cancer Cell Lines*

Cell type	Subtype	Designation	Origin	TNM	Stage	Gross morphology[a]
NSCLC	Adeno	Ma-10	Pleural effusion	T4N3M1	IV	4
	Adeno	Ma-24	Primary tumor	T3N2M1	IV	4
	Adeno	Ma-29	Pleural effusion	T4N3M0	IIIB	4
	Adeno	Ma-31	Primary tumor	T2N0M0	I	4
	Adeno	Ma-45	Pleural effusion	T4N0M0	IIIB	4
	Squamous	Ma-44	Primary tumor	T2N0M0	I	4
	Squamous	Ma-46	Primary tumor	T2N0M0	I	4
	Large	Ma-2	Pleural effusion	T4N2M1	IV	4
	Large	Ma-25	Pleural effusion	T4NxMx	IIIB	4
SCLC	Classic	N-230	Lymph node	—	—	2
	Classic	N-231	Lymph node	—	—	2
	Classic	H-209	Bone marrow	—	—	2
	Variant	N-417	Lymph node	—	—	3
	—	Ms-16	Primary tumor	—	—	2

*NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; adeno, adenocarcinoma; squamous, squamous cell carcinoma; large, large cell carcinoma.

[a]Gross morphology: 1, growing as tightly packed spherical aggregates of floating cells; 2, growing as relatively densely packed floating aggregates; 3, growing as very loosely adherent floating aggregates; 4, growing attached to the substrate.

samples of non-small cell lung cancer (NSCLC). Some small cell lung cancer (SCLC) lines expressed CD44s, but none of the SCLC human samples obtained during surgery or biopsy expressed CD44s, and none of the SCLC lines and human samples expressed CD44v, as shown by reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemistry [12,13]. Thus, there are discrepancies between SCLC lines and specimens in CD44s expression.

We examined the expressions of CD44s and each CD44v—variant exon 3 (v3), variant exon 5 (v5), variant exon 6 (v6), variant exon 7 (v7), variant exon 10 (v10)—molecules in cell lines of NSCLC and SCLC using RT-PCR, and the expressions of CD44 protein and v6 protein, which appeared to be related to cancer metastasis [14], in cell lines and human samples of NSCLC and SCLC, using immunohistochemistry.

MATERIALS AND METHODS

Human Lung Cancer Cell Lines

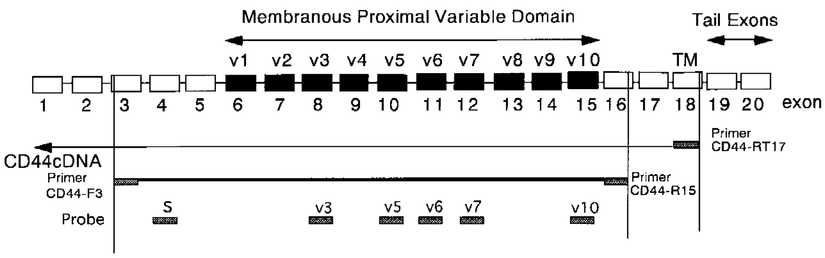
The human lung cancer cell lines used in this study include NSCLC; Ma-10, 24, 29, 31, and 45 (adenocarcinomas); Ma-44 and 46 (squamous cell carcinomas); Ma-2 and 25 (large cell carcinomas); and SCLC; N-230, N-231, H-209, N-417, N-417′ and Ms-16. The cell lines Ma-10, 24, 29, 31, 44, 45, and 46 and Ms-16 were obtained from the Prefectural Habikino Hospital (Osaka, Japan) [15,16]. Cell lines N-230, N-231, H-209, and N-417′ were obtained from A.F. Gazdar and H. Oie (National Cancer Institute [NCI]-Navy Medical Oncology Branch, National Institutes of Health [NIH], Bethesda, MD) through Dr. Shimosato, National Cancer Center Institute, Tokyo, Japan. Cell line N-417 was obtained directly from A F. Gazdar and H. Oie [17]. All NSCLC lines grew as adherent cultures in RPMI-1640 supplemented with 10% fetal calf serum (FCS). All SCLC lines except N-417 and N-417′ grew as suspension cultures in RPMI-1640 supplemented with 10% FCS (the N-417 and N-417′ cell lines partially adhered to the flasks.). The characteristics (histology, TNM factor, stage, and gross morphology) of the cell lines are summarized in Table I. The TNM factor, stage, and histology were determined at the time when each tumor sample was obtained from surgical specimens, biopsy specimens, and thoracic effusions to establish cell lines. We used the Union Internationale Contre le Cancer (UICC) TNM staging system (1987) [18]. Tumor histology was determined according to the WHO classification of lung tumors (1981) [19].

Tumor Samples

Twenty tumor samples were obtained from lung cancer patients during surgery at The Second Department of Surgery, School of Medicine, University of Tokushima. These samples consisted of 13 NSCLCs (six squamous cell carcinomas, seven adenocarcinomas) and seven SCLCs. All samples were frozen in O.C.T. compound (Miles, Elkhart, IN, USA) immediately after surgical excision and stored at −80°C until used.

RT-PCR Amplification of CD44

Total RNA was extracted from lung cancer cell lines by the single-step method with an acid guanidium thiocyanate–phenol–chloroform mixture [20]. RT-PCR analysis was performed as previously described [21,22]. The first strand of cDNA was synthesized from 1 μg of total RNA with CD44-specific oligonucleotide primer (Fig 1, primer RT-17). One microliter of the cDNA products was added to PCR reaction mixtures (10 μl) that contained PCR buffer (20 mM Tris-HCl, 50 mM KCl, 2 mM $MgCl_2$, 0.1 mg/ml bovine serum albumin [BSA]),



Primer CD44-RT17 :5'-TTGACTGCAATGCAAACTGC-3'
Primer CD44-F3 :5'-GACACATATTGCTTCAATGCTTCAGC-3'
Primer CD44-R15 :5'-TCAGATCCATGAGTGGTATGGGAC-3'
Probe CD44-S :5'-CCTGAAGAAGATTGTACATCAGTCACA-3'
Probe CD44-v3 :5'-CGTCTTCAAATACCATCTCAGCAGGC-3'
Probe CD44-v5 :5'-GAATGTGGGGTCTCTTCTTCCTCATG-3'
Probe CD44-v6 :5'-GGAAGAAACAGCTACCCAGAAGGAACAGTG-3'
Probe CD44-v7 :5'-GCTCATACCAGCCATCCAATGCAA-3'
Probe CD44-v10 :5'-GATAAGGAACGATTGACATTAGAGTTGG-3'
Primer β-actin-RT :5'-ATCACGATGCCAGTGGTACG-3'
Primer β-actin-PCR5' :5'-GTCAGAAGGATTCCTATGTG-3'
Primer β-actin-PCR3' :5'-GCCTGGATAGCAACGTACAT-3'

Fig. 1. Schematic representation of the human CD44 gene. The human CD44 gene consists of constitutively splicing exons (unfilled boxes) and alternatively splicing exons (filled boxes). Reverse transcription (RT) was performed with the primer CD44-RT17. Polymerase chain reaction (PCR) was performed by primer CD44-F3 and R15. Each probe (S, v3, v5, v6, v7, v10) was used in the hybridization. The sequences of the primers and probes used in this study are shown.

200 μM of each dNTP, 0.25 units of *Taq* polymerase, and 1 μM CD44 oligonucleotide primers (Fig. 1, primer F3 and R15). The PCR conditions used were 94°C, 0.5 min: 60°C, 0.5 min: 72°C, 1 min for 30 cycles. We decided that 30 cycles was the most suitable number of cycles for this study [22]. The PCR products were separated by electrophoresis on 1.5% agarose gels and visualized under ultraviolet (UV) light after ethidium bromide staining. The RT-PCR of β-actin for each cell line was used as a control. Expression levels of CD44 molecules, which were either the same or more than that of β-actin, were defined as ''strong,'' and expression levels which were less than that of β-actin were defined as ''weak'' (Fig. 2a, Table II). The β-actin specific oligonucleotide primers for RT or PCR are shown in Figure 1. The PCR products were transferred to nylon membranes and hybridized with 5′-end-labeled oligonucleotide probes complementary to CD44 exon 4, exon v3, v5, v6, v7, and v10. Each probe used in the hybridization is shown in Figure 1. Filters were washed at room temperature (2× SSC, 0.1% SDS) for 5 min, 3 times and at 50°C (0.1× SSC, 0.1% SDS) for 15 min, twice before autoradiography. To ensure consistency of assays, the RT-PCR of the CD44 and β-actin gene was performed in two independent studies. Our previous study showed that all normal lung tissues expressed CD44s and that one-half expressed CD44 v10, but rarely expressed CD44v except v10 [22].

Immunohistochemical Staining

Immunohistochemical staining was performed, as previously described [22]. All NSCLC cell lines grew on the slides at the bottom of the culture. All SCLC cell lines were pipetted onto the slides and gently smeared after centrifugation. The slides with cell lines and frozen sections (6 μm) of surgically resected samples were fixed in ice-cold acetone for 10 min after 2 h of air drying, washed in a TBS solution (0.05 M Tris, 0.15 M NaCl, pH 7.6), and preincubated with BSA for 5 min. They were removed by tapping and then incubated with primary antibodies (SFF-2: murine monoclonal antibodies against human standard CD44 or VFF-7: murine monoclonal antibodies against an epitope encoded by exon v6 of the human variant CD44, Bender MedSystems, Vienna, Austria) [9,11] at 4°C for 12–16 h. A 1 : 100 dilution of these antibodies was considered optimal following titration experiments using a panel of control tumors. The sections were incubated with the secondary biotinylated antibody for 15 min to permit formation of the streptavidin–biotin–peroxidase complex. Visualization of the immunocomplex was performed by the immunoperoxidase-3,3′-diaminobenzidine method for 2 min [23] (Dako LSAB Kit™, Dako, Carpinteria, CA); the reaction was then stopped in H_2O. The cells were counterstained with hematoxylin, mounted with Malinol, and viewed under a microscope. Under a light microscope, we independently evaluated 1,000 tumor cells under high magnification (K. Kondo and T. Miyoshi, unpublished observations). Cases with more than 50% positive tumor cells were defined as ''diffusely stained,'' those with less than 50% were ''focally stained,'' and those without positive tumor cells were ''not stained.'' The immunostaining of CD44s and v6 in cell lines and tumor samples was twice performed independently. Our previous study showed that all normal lung specimens (type I and II pneumocytes, interstitial tissues, and macrophages) were stained by anti-CD44s antibody. Normal lung tissues were little (or few) stained by anti-CD44v6 antibody, but part of type II pneumocytes were stained [22].

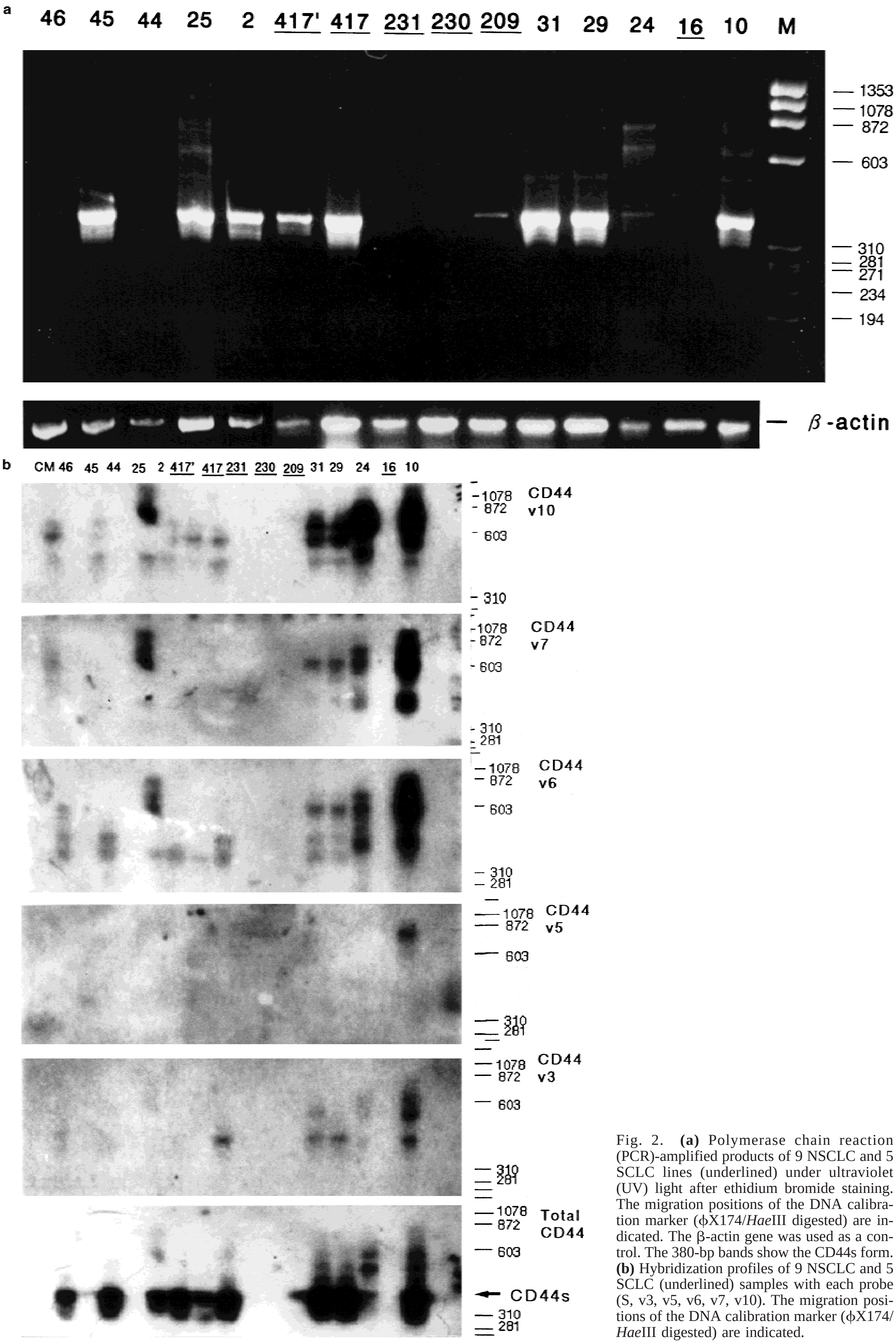


Fig. 2. **(a)** Polymerase chain reaction (PCR)-amplified products of 9 NSCLC and 5 SCLC lines (underlined) under ultraviolet (UV) light after ethidium bromide staining. The migration positions of the DNA calibration marker (ϕX174/*Hae*III digested) are indicated. The β-actin gene was used as a control. The 380-bp bands show the CD44s form. **(b)** Hybridization profiles of 9 NSCLC and 5 SCLC (underlined) samples with each probe (S, v3, v5, v6, v7, v10). The migration positions of the DNA calibration marker (ϕX174/*Hae*III digested) are indicated.

TABLE II. Expression of CD44s and CD44v in Lung Cancer Cell Lines, as Shown by RT-PCR or Immunostaining*

		RT-PCR[a,b]						Immunostain[c]	
Cell type	Designation	CD44s	v3	v5	v6	v7	v10	CD44	v6
NSCLC	Ma-10	++	+	+	+	+	+	++	+
	Ma-24	+	+	−	+	+	+	++	+
	Ma-29	++	+	−	+	+	+	++	+
	Ma-31	++	+	−	+	+	+	++	+
	Ma-45	++	−	−	+	−	+	++	−
	Ma-44	−	−	−	−	−	−	−	−
	Ma-46	+	−	−	−	−	−	nd	nd
	Ma-2	++	−	−	−	−	−	++	−
	Ma-25	++	+	−	+	+	+	++	++
SCLC	N-230	−	−	−	−	−	−	−	−
	N-231	−	−	−	−	−	−	−	−
	H-209	+	−	−	−	−	−	+	−
	N-417	++	+	−	+	−	+	++	+
	Ms-16	−	−	−	−	−	−	−	−

RT-PCR, reverse transcription-polymerase chain reaction; nd, not done; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.
*As the signal level of all CD44v bands was weaker than that of β-actin PCR bands (Fig. 2a), the cell line with each CD44v expression was shown as ''+''.
[a]Degree of expression: −, none; +, weak; ++, strong.
[b]v3, v5, v6, v7, v10, variant forms of CD44.
[c]Degree of immunostaining: −, none; +, focal; ++, diffuse.

RESULTS

RT-PCR of CD44 in Lung Cancer Cell Lines

The PCR amplification products from lung cancer cell lines were visualized under UV light after ethidium bromide staining (Fig. 2a). Each lung cell line was tested using β-actin PCR before CD44s amplification to check for the quality and abundance of RNAs. Eight of nine NSCLC cell lines (89%) were amplified for CD44 to varying degrees. Six NSCLC lines strongly expressed CD44s (380 bp), whereas two of five SCLC cell lines (40%) were amplified. The N-417 line strongly expressed CD44s. The signal level of all CD44v bands was weaker than that of β-actin PCR bands.

Expression of the CD44 Standard or Variant Exons (v3, v5, v6, v7, v10) in NSCLC Cell Lines

In NSCLC cell lines, 8 of 9 (89%) expressed the CD44 standard form, though 2 cell lines (Ma-24, 46) had weak signals (Fig. 2b, Table II). Six of nine cell lines (67%) expressed various CD44 variant forms. Only the Ma-10 line expressed all the variant exons (v3, 5, 6, 7, and 10) we examined. Four lines (Ma-24, 25, 29, and 31) expressed some variant exons, including v3, v6, v7, and v10. The Ma-45 line expressed v6 and v10.

All five adenocarcinoma cell lines expressed variant exons, but both the squamous cell carcinoma cell lines did not express them. In large cell carcinoma cell lines, one expressed variant exons, but the other did not. In the advanced-stage cases (stages IIIB and IV), five of six expressed variant exons. But only one of three early-stage cases (stage I) expressed them.

Expression of the CD44 Standard or Variant Exons (v3, v5, v6, v7, v10) in SCLC Cell Lines

In SCLC cell lines, two of five cell lines expressed the CD44 standard form (Fig. 2b, Table II). The H-209 line weakly expressed the standard form, but not the variant form. The N-417 line expressed the standard form and the variant form (v3, v6, and v10). Only the N-417 line belonged to the variant subtype, which grew as very loosely adherent floating aggregates.

Immunohistochemistry of the CD44 and Variant Exon 6 in Lung Cancer Cell Lines

To determine the expression of the CD44 and variant 6 form in lung cancer cell lines, immunohistochemical staining by anti-CD44s (which recognized both CD44s and CD44v) and anti-CD44v6 antibodies (which recognized only CD44v6) was carried out with acetone-fixed specimens from eight NSCLC cell lines and five SCLC cell lines (Fig. 3, Table II). Seven of eight NSCLC lines were stained by anti-CD44s antibodies. These seven lines concurred with the cell lines shown to express CD44s by RT-PCR. Five of six lines that expressed CD44v6, as shown using RT-PCR, were stained by anti-CD44v6 antibodies. Only the Ma-25 cell line was diffusely stained (Fig. 3a,b).

The N-417 line (variant type), which was shown to express CD44s and v6 using RT-PCR, was stained by anti-CD44s and v6 antibodies (Fig. 3c,d). Only one line (H-209) in the classic type of SCLC, which showed weak expression of CD44s using RT-PCR, was focally stained by CD44s antibodies. The other three classic SCLC lines

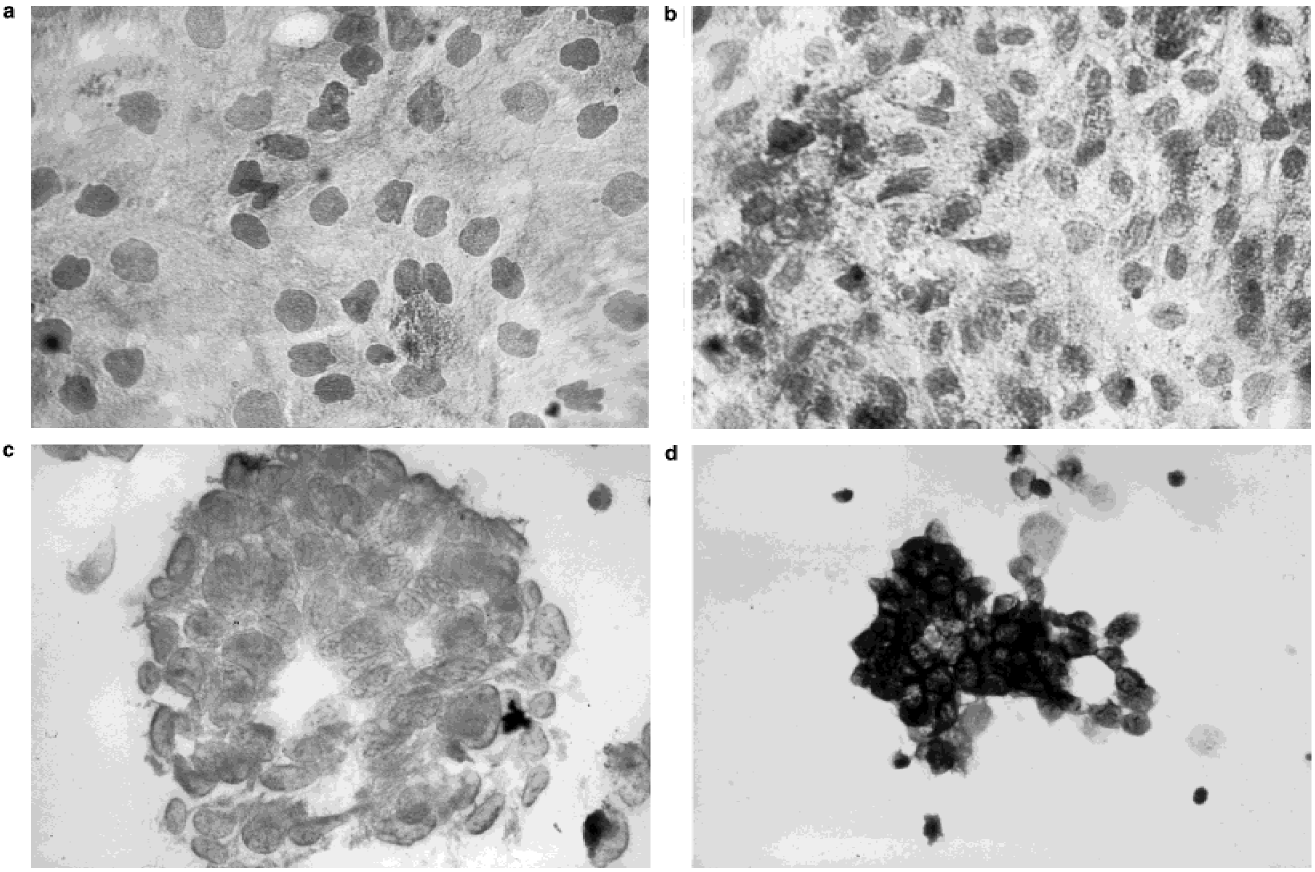


Fig. 3. Immunostaining of the NSCLC and SCLC lines with anti-CD44s and v6 antibodies. The Ma-25 line (NSCLC) was strongly stained by both anti-CD44s **(a)** and v6 **(b)** antibodies. The N-417 line (SCLC, variant subtype) was strongly stained by both anti-CD44s **(c)** and v6 **(d)** antibodies.

were not stained by anti-CD44s antibodies. All four classic SCLC lines were not stained by anti-CD44v6 antibodies.

Immunohistochemistry of CD44 and Variant Exon 6 in Lung Cancer Samples

Immunohistochemical staining of CD44 and v6 was carried out with OCT compound-frozen specimens from 13 NSCLC and 7 SCLC tumors (Fig. 4, Table III). All 13 NSCLC were diffusely stained by anti-CD44s antibodies. Not only cancer cells but also noncancerous components were diffusely stained (Fig. 4a,c). Nine of 13 NSCLC (69%) were stained by anti-CD44v6 antibodies. Cancer cells were focally or diffusely stained, but noncancerous components were not stained (Fig. 4b,d). Five of six squamous cell carcinomas (83%) were diffusely stained by anti-CD44v6 antibodies. However, in adenocarcinoma, although four of seven cases (57%) were stained, only one case was diffusely stained (Table III).

Four of seven SCLC (57%) were focally stained by anti-CD44s antibodies (Fig. 4e, Table III). Although the immunoreactivity of SCLC was less than that of NSCLC, cancer cell membranes of SCLC were clearly stained (Fig.4e). None of seven SCLC was stained by anti-CD44v6 antibodies (Fig. 4f, Table III).

DISCUSSION

The CD44 standard and variant forms participated in cell–cell and cell–matrix interactions such as lymphocyte recirculation and prothymocyte homing, hematopoiesis, lymphocyte and monocyte activation, cell migration, and metastasis [24].

This study demonstrated that some SCLCs obviously expressed CD44s. Penno et al. [12] reported that three of seven SCLC lines (43%) obviously expressed the CD44 molecule, but that none of the nine SCLC surgically resected cases were immunopositive for CD44. There was a discrepancy in CD44 expression between SCLC cell lines and SCLC samples obtained at surgery in Penno's study.

Subsequent studies supported this discrepancy. Jackson et al. demonstrated that all three SCLC lines examined strongly expressed CD44s using RT-PCR[13]. Ariza et al. [25] reported no immunoreactivity for CD44s in all 11 SCLC biopsy specimens. However, in our study we found that two of five SCLC lines expressed CD44s at

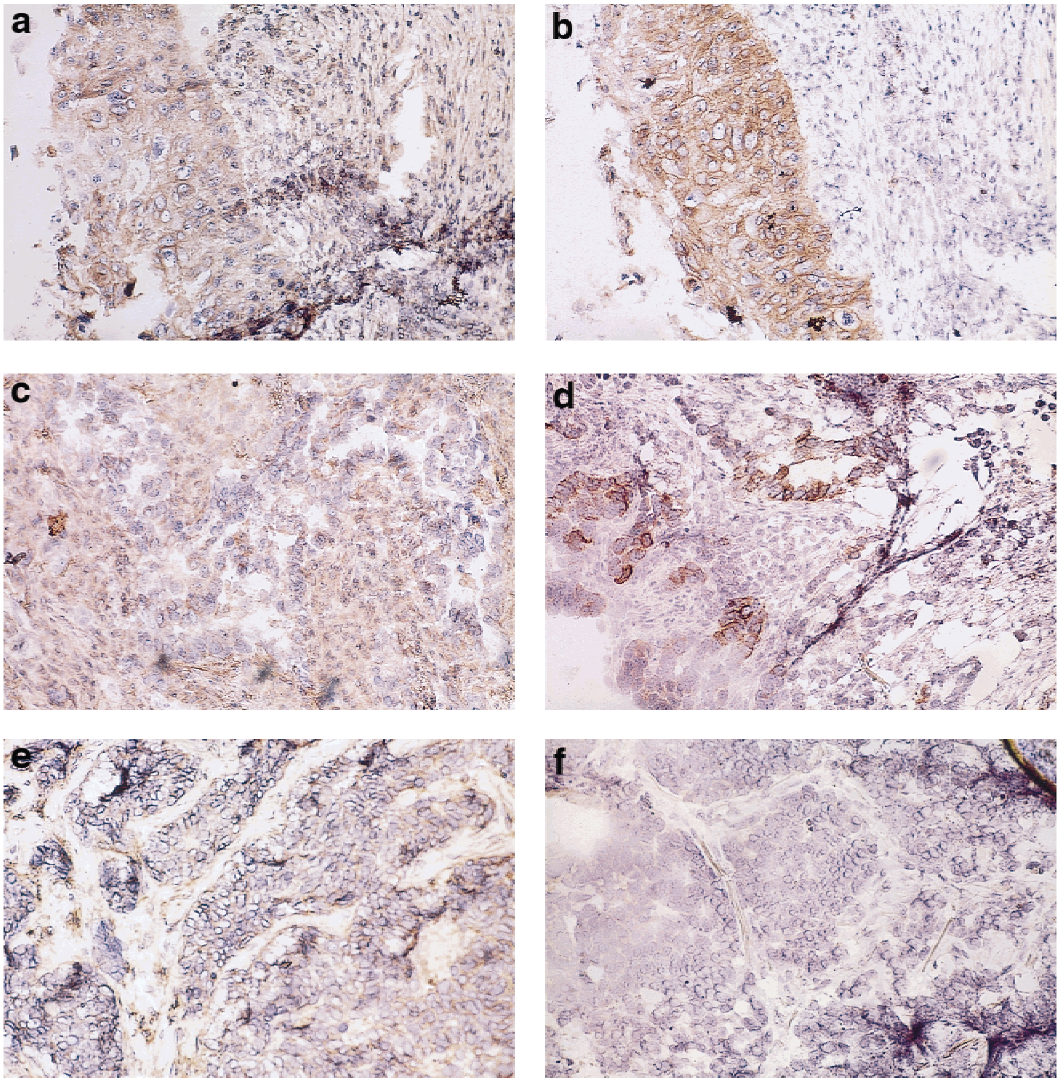


Fig. 4. Immunostaining of NSCLC and SCLC surgically resected samples using anti-CD44s and v6 antibodies. Not only tumor cells, but also stromal cells in squamous cell carcinoma and adenocarcinoma, were strongly stained by anti-CD44s antibodies **(a,c)**. In SCLC, almost all stromal cells were stained by anti-CD44s antibodies, but the cell membranes of tumor cells were weakly stained **(e)**. The tumor cells in squamous cell carcinoma and adenocarcinoma were selectively stained by anti-CD44v6 antibodies, but other cells, including stromal cells, were never stained **(b,d)**. Neither tumor cells or stromal cells in SCLC were stained by anti-CD44v6 antibodies **(f)**.

TABLE III. Expression of CD44 and v6 in Surgically Resected Lung Cancer Samples*

		No. of CD44 positives			No. of CD44v6 positives		
Histology	No. of cases	Focal	Diffuse	Total	Focal	Diffuse	Total
SCLC	7	4	0	4 (57%)	0	0	0 (0%)
NSCLC	13	0	13	13 (100%)	3	6	9 (69%)
Squamous	6	0	6	6 (100%)	0	5	5 (83%)
Adeno	7	0	7	7 (100%)	3	1	4 (57%)

*NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; adeno, adenocarcinoma; squamous, squamous cell carcinoma.

the mRNA and protein levels and that four of seven SCLC surgically resected samples were focally stained by anti-CD44s antibodies. The discrepancy of CD44s immunoreactivity in SCLC samples between our study and the studies conducted by Penno et al. [12] or Ariza et al. [25] may to be attributable to differences in fixation methods of specimens and anti-CD44 antibody. In particular, although they used formalin-fixed, paraffin-

embedded sections for immunohistochemistry of CD44s, we used frozen sections. It has been reported that formalin-fixation methods weaken the antigenicity [26,27]. We conclude that the results of our immunohistochemical analysis with frozen sections are more reliable than those with formalin-fixed sections and that the immunoreactivity of in vitro SCLC cells by anti-CD44s antibodies coincided with that of in vivo SCLC cells.

SCLC rarely expresses CD44v. Jackson et al. [13] demonstrated that 3 SCLC lines did not express CD44v using RT-PCR. Ariza et al. [25] found that none of 11 SCLC samples obtained by biopsy expressed CD44 v3 or v6 by immunostaining. Our study showed that only one of 5 SCLC lines expressed CD44v and that none of seven SCLC surgically resected samples were stained by anti-CD44v6 antibodies.

However, NSCLC always expresses CD44s and frequently CD44v. We detected CD44s in 8 of 9 NSCLC lines, and various CD44v in six of nine NSCLC lines using RT-PCR. The immunostaining findings from the NSCLC cell lines by human anti-CD44s and CD44v6 antibodies were in agreement with the RT-PCR results. All 13 NSCLC surgically resected samples expressed CD44s, as shown using immunostaining, and 9 of 13 NSCLC samples expressed CD44v6. The findings obtained in the present study concurred with the results of other studies [13,25,28]. Our previous study demonstrated that all normal lung tissues expressed CD44s, and that half of them expressed CD44 v10 [22].

In tumor cells, three alterations of CD44 expression have been observed: downregulation of CD44s and CD44v in neuroblastoma [29], downregulation of only CD44v in endometrial carcinoma, squamous cell carcinoma in the oropharynx [30,31], and upregulation of CD44s or CD44v in many cancers, such as colorectal and breast cancer [7–11]. We speculate that CD44s and CD44v expression in SCLC is downregulated as in neuroblastoma, and that CD44v expression in NSCLC is upregulated as in many cancers compared with normal lungs. The downregulation of CD44 molecules in neuroblastoma is related to the metastatic potential [32]. It was reported that SCLC is characterized by an extremely aggressive growth pattern with early metastatic spread compared with NSCLC [33]. We suggest that downregulation of CD44 molecules plays a role in the high metastatic potential of SCLC. In this respect, it is necessary to clarify whether SCLC with CD44s expression has a lower metastatic potential.

The N-417 line (SCLC) expressed CD44s and some CD44v, as shown using RT-PCR and immunostaining. In previous studies, there was no SCLC which expressed CD44v [12,13,25]. Indeed, N-417 belonged to a variant subtype of SCLC, and grew very loosely in adherent floating aggregates and partially grew attached to the substrate. In contrast (or contractively), four other SCLCs lines that expressed little or no CD44 belonged to the classic SCLC subtype and grew as relatively densely packed floating aggregates. All eight NSCLC, seven of which expressed CD44, grew attached to the substrate. Carney et al. [34] subgrouped SCLC lines into classic and variant SCLC lines and showed that variant SCLC was borderline between classic SCLC and NSCLC in both biomarkers and growth patterns. Penno et al. [12] demonstrated that the SCLC cell line (H-82) infected with the v-Ha-*ras* oncogene lost the typical aggregated growth pattern of SCLC and grew in monolayers, typical of NSCLC. SCLC cell lines infected with v-Ha-*ras* began to express CD44, although the parent cell lines did not express CD44. We suggest that CD44 expression of NSCLC and N-417 may be related to the growth pattern attaching to the substrate. CD44 downregulation of SCLC may be related to floating growth without attachment to substrate. Decreased adhesion of tumor cells to the substrate in SCLC would favor their emigration from local sites and would aid dissemination.

CONCLUSION

CD44 expression was repressed in SCLC. However, not only CD44s but also CD44v were frequently expressed in NSCLC. Downregulation of CD44 molecules might play a role in the high metastatic potential of SCLC.

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